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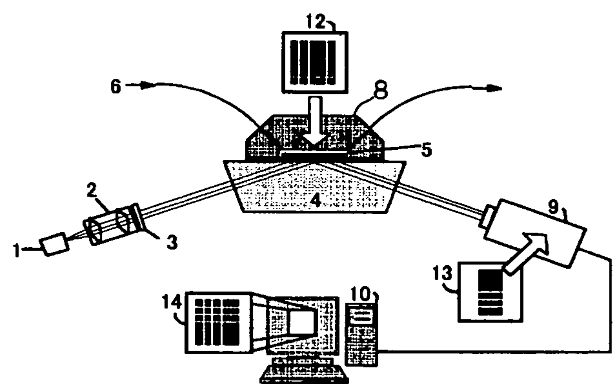
最終頁に続く

(54)【発明の名称】2次元イメージング表面プラズモン共鳴測定装置および測定方法

(57)【要約】

【課題】　アスペクト比を補正する機能を有し、被測定物質の実際のアスペクト比に合致した測定データが得られる2次元表面プラズモン共鳴測定装置を提供するとともに、さらに、抗原抗体反応などの生体物質や遺伝子の多チャンネル測定、生物や細胞の物質の代謝などの様子を測定する方法を提供する。

【解決手段】　被測定物質に接する金属薄膜を表面上に備えたプリズムと、光源からの光をp偏光光の平行な光束としてプリズム側から入射させる入光手段と、金属薄膜からの表面プラズモン共鳴現象による吸収を含んだ反射光の2次元の受光光量を測定する受光手段とを有し、前記入光手段により光束を前記金属薄膜の面に対し斜めに入射することによる、反射光のアスペクト比の被測定物質の金属薄膜への接触面におけるアスペクト比に対する歪みを補正する手段を備えたことを特徴とする2次元イメージング表面プラズモン共鳴測定装置、およびそれを用いた測定方法。



【特許請求の範囲】

【請求項１】　被測定物質に接する金属薄膜を表面上に備えたプリズムと、光源からの光をｐ偏光光の平行な光束としてプリズム側から入射させる入光手段と、金属薄膜からの表面プラズモン共鳴現象による吸収を含んだ反射光の２次元の受光光量を測定する受光手段とを有し、前記入光手段により光束を前記金属薄膜の面に対し斜めに入射することによる、反射光のアスペクト比の被測定物質の金属薄膜への接触面におけるアスペクト比に対する歪みを補正する手段を備えたことを特徴とする２次元イメージング表面プラズモン共鳴測定装置。

【請求項２】　前記アスペクト比を補正する手段が、コンピュータなどの演算処理手段からなる、請求項１に記載の測定装置。

【請求項３】　前記アスペクト比を補正する手段が、反射光をアナモフィックレンズを使用して補正する手段からなる、請求項１に記載の測定装置。

【請求項４】　前記アスペクト比を補正する手段が、反射光をアスペクト比補正用のプリズムを使用して補正する手段からなる、請求項１に記載の測定装置。

【請求項５】　前記アスペクト比を補正する手段が、２次元の受光光量を測定する素子を測定面に平行に保ったまま、反射光の角度に応じて移動させる機構を有する手段からなる、請求項１に記載の測定装置。

【請求項６】　前記アスペクト比を補正する手段が、アスペクト比の補正量を任意に変えることのできる手段からなる、請求項１～５のいずれかに記載の測定装置。

【請求項７】　光源に、光ファイバにより伝播された光が用いられている、請求項１～６のいずれかに記載の測定装置。

【請求項８】　角度を走査して得られた複数の画像データにおいて、ある一点もしくは複数の点を指定し、その点における反射強度を抽出、グラフとして描画することのできるコンピュータなどの演算処理装置を有する、請求項１～７のいずれかに記載の測定装置。

【請求項９】　被測定物質の任意の領域を測定するため、被測定物質、あるいは、プリズムと被測定物質の両方を測定面に平行に移動することができる機構を有する、請求項１～８のいずれかに記載の測定装置。

【請求項１０】　請求項１～９のいずれかに記載の２次元イメージング表面プラズモン共鳴測定装置を用い、金属薄膜を区切り、分割した各金属薄膜上に異なる抗体や酵素等を固定化したセンサ膜を作製し、同一の被測定物質をセンサ膜に接触させることにより、または、分割した各金属薄膜に同じ抗体や酵素等を固定化したセンサ膜を作製し、それぞれに異なる被測定物質を接触させることによって、同時に多種類の化学物質の定量・定性を行うことを特徴とする、２次元イメージング表面プラズモン共鳴測定方法。

【請求項１１】　遺伝子を固定化したセンサ膜を作製し、被測定物質として各種の化学物質を導入することによって、遺伝子に影響を与える化学物質を検知する、請求項１０の測定方法。

【請求項１２】　既知の配列を持った遺伝子を固定化したセンサ膜を作製し、被測定物質として未知の配列を持った遺伝子を導入することによって、反応を起こした金属薄膜表面での座標から未知の遺伝子の配列を検知する、請求項１０の測定方法。

【請求項１３】　生体物質を固定化したセンサ膜を作製し、被測定物質として各種の化学物質を導入することによって、生体物質に影響を与える化学物質を検知する、請求項１０に記載の測定方法。

【請求項１４】　金属薄膜上の被測定物質の屈折率分布を測定する、請求項１０～１３のいずれかに記載の測定方法。

【請求項１５】　請求項１～９のいずれかに記載の２次元イメージング表面プラズモン共鳴測定装置を用い、金属薄膜上に生物や細胞が取りこむ物質または排出する物質に感応する抗体や酵素等を配置もしくは固定化したセンサ膜を作製し、その上に生物や細胞を置くことによって、その代謝の様子を測定することを特徴とする、２次元イメージング表面プラズモン共鳴測定方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、光学系を用いて被測定溶液中の特定物質を定量あるいは定性的に測定する表面プラズモン共鳴測定装置および測定方法に関するものである。詳しくは、被測定物質に接した金属薄膜での表面プラズモン共鳴現象を利用して、液体やガスなどの被測定物質の屈折率変化を検知し定性・定量測定を行うものであり、金属薄膜面の２次元測定を可能とする測定装置、および、その測定方法に関する。

【０００２】

【従来の技術】従来、化学プロセス計測、環境計測や臨床検査等で呈色反応や免疫反応を利用した測定が行われている。しかしこのような測定方法では、被測定物質をサンプル抽出する必要があるほか、煩雑な操作や標識物質を必要とするなどの問題がある。これに対し、標識物質を必要とすることなく、高感度で被測定物質中の化学物質の定性・定量測定の可能なセンサとして光励起表面プラズモン共鳴現象を利用したセンサが提案・実用化されている。以下、表面プラズモン共鳴（Ｓｕｒｆａｃｅ　Ｐｌａｓｍｏｎ　Ｒｅｓｏｎａｎｃｅ）をＳＰＲと略して用いることもある。

【０００３】ＳＰＲセンサを構成するシステムとしては、例えば図１に示すように、光源１から発した光をｐ偏光光のみを通す偏光板２、及び集光レンズ３を通し高屈折率プリズム４にある入射角を持って入射させ、被測定物質６に接するセンサ膜を有した金属薄膜５に照射し、金属薄膜５からの反射光の強度変化をプリズム４を

通して光電子検出器７で検出するシステムが一般的である。

【０００４】光源１から発した光は、プリズム４と金属薄膜５の界面でエバネッセント波を生起させ、その波数は次式により定義される。

$k_{ev}=k_p n_p \sin\theta$

ここで、k_p は入射光の波数、n_p はプリズムの屈折率、θ は入射角である。

【０００５】一方、金属薄膜５の表面では、表面プラズモン波が生じ、その波数は次式により定義される。

$k_{sp}=(c/\omega)\cdot\sqrt{(\varepsilon n^2/(\varepsilon+n^2))}$

ここで、ｃは光速、ω は角振動数、ε は金属薄膜の誘電率、ｎは被測定物質の屈折率である。

【０００６】このエバネッセント波と表面プラズモン波の波数が一致する入射角 θ のとき、エバネッセント波は表面プラズモンの励起に使われ、例えば図２に示すように、反射光として観測される光量が減少する。

【０００７】ＳＰＲ現象は、プリズム・金属薄膜に接した被測定物質の屈折率に依存するために、例えば、被測定物質を水とした場合、図２に示すようにある一定の角度で極小を持つ曲線として検出することができ、被測定物質の化学的濃度変化による屈折率変化等を測定するばかりか、金属薄膜上に抗体等を固定化することにより、抗原と結合した抗体の屈折率変化を測定することにより、特定物質の定量を行うことができる。

【０００８】しかし、近年、被測定物質の屈折率分布の測定、および、多試料の測定への対応が要求されている。

【０００９】そこで、被測定物質の屈折率分布を測定するために、図３に示すように、光源１からの光を偏光板２を通し入射された光をレンズ３で平行光へと変換し、プリズム４を通して領域を持った金属薄膜５上の被測定物質６のＳＰＲ現象をＣＣＤ（ｃｈａｒｇｅ－ｃｏｕｐｌｅｄ　ｄｅｖｉｃｅ）カメラ７で測定する装置が提案されている。もちろん、平行光はひとつの入射角しか持たないために、入射角と反射角とを常に同期して駆動しうる機構が必要である。しかし、図３のようなシステムの装置では、ある角度を持って反射した光を観測するために、実際の測定面、例えば図４（ａ）に示すように金属薄膜５ａ（例えば、金の薄膜）が２次元配列された計測面と、観測されるデータ取得面５ｂ（図４（ｂ））とではアスペクト比（縦横比）が異なるという問題があった。

【００１０】また、多試料の測定への対応については、入射光を並列に並べ、試料を入れるためのセルを同じく並列に並べることによって、数個の試料を同時に測定する装置が知られているが、それ以上の同時測定は不可能であり、限界がある。

【００１１】

【発明が解決しようとする課題】本発明はこのような現状に鑑みてなされたものであり、その課題は、アスペクト比を補正する機能を有することを特徴とし、被測定物質の実際のアスペクト比に合致した測定データの得られる２次元イメージング表面プラズモン共鳴測定装置を提供することにある。さらに、本発明の別の課題は、本発明による装置を使用した抗体抗原反応などの生体物質や遺伝子の多チャンネル測定、生物や細胞の物質の代謝などの様子を測定する方法を提供することにある。

【００１２】

【課題を解決するための手段】上記課題を解決するために、本発明は、２次元イメージング表面プラズモン共鳴測定装置において得られる画像が、被測定物質のアスペクト比と同じに得られるようにアスペクト比補正機能を加えたものである。すなわち、本発明に係る２次元イメージング表面プラズモン共鳴測定装置は、被測定物質に接する金属薄膜を表面上に備えたプリズムと、光源からの光をｐ偏光光の平行な光束としてプリズム側から入射させる光学手段と、金属薄膜からの表面プラズモン共鳴現象による吸収を含んだ反射光の２次元の受光光量を測定する受光手段とを有し、前記入光手段により光束を前記金属薄膜の面に対し斜めに入射することによる、反射光のアスペクト比の被測定物質の金属薄膜への接触面におけるアスペクト比に対する歪みを補正する手段を備えたことを特徴とするものからなる。

【００１３】アスペクト比補正には、受光手段の光検出器で検出されたデータをコンピュータなどの演算装置を用いて補正を行う演算方法と、反射光が光検出器に入る前に光学的手段で補正を行うものと、光検出器そのものの位置を常に補正しうるように駆動させる機械的方法が考えられる。

【００１４】このようなアスペクト比の補正によって、被測定物質と同じアスペクト比の画像が得られ、２次元イメージセンサとしての応用が可能となる。

【００１５】本発明に係る２次元イメージング表面プラズモン共鳴測定方法は、上記のような２次元イメージング表面プラズモン共鳴測定装置を用い、金属薄膜を区切り、分割して各金属薄膜上に異なる抗体や酵素等を固定化してセンサ膜を作製し、同一の被測定物質をセンサ膜に接触させることにより、または、分割した各金属薄膜に同じ抗体や酵素等を固定化したセンサ膜を作製し、それぞれに異なる被測定物質を接触させることによって、同時に多種類の化学物質の定量・定性を行うことを特徴とする方法からなる。すなわち、多チャンネルセンサとして使用できるように金属薄膜に区切りを形成し、それぞれに区切られた金属薄膜に、異なる抗体や酵素等を固定化したり異なる試料を乗せられるようにしたものである。

【００１６】

【発明の実施の形態】以下に、本発明の各実施の形態を、図面を参照しながら説明する。本発明の第一の特徴

はアスペクト補正機能を付与した点である。演算方式によるアスペクト比補正機構として、図５にその例を示す。金属薄膜５からＳＰＲ吸収を含んだアスペクト比が正常でない反射光を一旦ＣＣＤカメラ９などで検出し、検出した画像をコンピュータ１０などの演算処理装置で適切なアスペクト比に補正する。セル８内の試料６（被測定物質）の実際の計測面１２に対し、ＣＣＤカメラ９で観測されるデータは画像１３のように歪むが、コンピュータ１０によって実際の計測面１２と同じ画像１４にアスペクト比が補正される。ただし、この方法ではＣＣＤの画素密度により補正時に画像が粗くなることがあり、適切な画像の補完処理が必要となる場合がある。

【００１７】光学的手段によるアスペクト比補正機構として、シリンドリカルレンズ、もしくはそれを組合せたアナモフィックレンズ、プリズムを組合せたシステム等を使用できる。図６にそのシステムの例を示す。金属薄膜５から反射されたアスペクト比の正常でない光束を、アナモフィックレンズを使用したアスペクト比補正機構、あるいは補正用のプリズムを組合せ使用したアスペクト比補正機構１１を通してＣＣＤカメラ９などで検出することにより、アスペクト比が実際の試料と同じ画像１４を得ることができる。

【００１８】以上の２つのシステムでは、入射角度によって反射光のアスペクト比が異なるために、その補正値を自由に変えられることが必要となってくる。

【００１９】機械的方法によりアスペクト比を校正する方法として、例えば、図７、図８に示すような機構を使用できる。図において、入射光側では、光源１、レンズ２、偏光子３はアーム１５に取りつけられている。出射光側にはアーム１６が取りつけられており、両アーム１５、１６には、２本のアーム１７がジョイント部１８で連結されたリンク機構からなる補助機構が取りつけられており、入射光に対して、アーム１６が常に出射光に沿って駆動するようになっている。そして、さらにアーム１９を取りつけることができる。このアーム１９は金属薄膜面に常に平行であり、アーム１９の出射光側に２次元の受光素子７（受光器）を取りつけてある。各部のジョイント部分は可動でアーム１９とアーム１５の接合部は、アーム１９に細長い穴があいておりスライド可能となっている。アーム１７のジョイント部１８は試料に対して垂直なガイド上を動く。この方法によれば、図７、図８に示すように入射光の角度を変えても、受光素子７は常に金属薄膜面に平行になり、入射角度の変化に伴う出射光の角度変化にも追従していることがわかる。この受光素子７上には特別なアスペクト比変換機構なしに常に正常なアスペクト比を持った画像を得ることができる。

【００２０】本発明では、光源として光ファイバを用いることができる。単なるＬＥＤ光源やＬＤ光源では出射光量のプロファイルが均一でなく、金属薄膜に入射する

とき明るさに斑ができてしまうという欠点があった。一度、光ファイバに光を通すことによって、ある程度均一な出射光量のプロファイルが得られ、均一な光束として金属薄膜面に光を当てることが可能となった。また、角度誤差のない平行光を得るためには点光源が必要であるが、光ファイバの光出射端を光源として選択すれば、目的に応じてコア径を数μｍから数百μｍまで選択することができる。

【００２１】また、本発明においては、図９～図１１に示すような工夫を加えることもできる。図９においては、例えば単色平行光を出射する熱的光源３１からの平行光を偏光子３２を通し、プリズム３３を通した金属薄膜３４からの反射光も偏光子３５を通して、ＣＣＤカメラ３６で画像を得る。偏光子３２、３５を組合せることによって解像度を上げることが可能になる。

【００２２】図１０においては、白色平行光を出射する熱的光源４１を用い、白色光を使用することによりスペクトル変化を見ることができるようにしてある。プリズム４２を通して金属薄膜４３からの反射光を、２次元画像分光システム４４やイメージング分光器とピエゾ駆動を組合せて観測することにより、取得されるデータのスペクトル変化を見ることができる。

【００２３】図１１においては、例えば半導体レーザ光源５１から格子状のレーザビーム５２を出射し、プリズム５３を通して金属薄膜５４からの反射光をＣＣＤカメラ５５で画像として得るようにしてある。格子状のレーザビーム５２を、例えば、マトリックス状に区画された各金属薄膜５４に対し個々に当てることにより、多チャンネルの同時測定が可能になる。

【００２４】本発明は、上記のような２次元イメージング表面プラズモン共鳴測定装置を使ったマルチチャンネルセンサとしての測定方法を提供する。その方法は、例えば、金属薄膜を分割した上に、それぞれ異なる物質に感応する抗体や酵素等を固定化し、一度に多種類の物質を定性・定量できるというものである。もしくは、分割した金属薄膜上に同じ抗体や酵素等を固定化しておき、異なる被測定物質をそれぞれに加えて定性・定量を行うものである。

【００２５】測定に用いる金属薄膜は、例えば図１２に示すような構造をしており、分割された金属薄膜２１が各々独立して透明体２０上に配置しているものや、金属薄膜２１を高分子２２などで区切る構造などに構成できるが、その形状、材質等は特に限定されるものではない。

【００２６】金属薄膜の分割の方法は、（１）金属薄膜を付けるときにマスクを使用する、（２）金属薄膜の上に光感応性樹脂などの高分子でパターンを作製する、（３）あらかじめパターンのついた高分子シートを貼り付ける、などの方法が考えられるが、それぞれの露出した金属薄膜に異なる抗体や酵素等を固定化したり、異な

る被測定物質を導入することを考えると、金属薄膜の間には区切りがあったほうが望ましい。

【００２７】以上のように作製した区切られた金属薄膜には、図１３に示すように異なった抗体や酵素等２３を固定化し、さらに被測定物質２４を加えることにより、試料中に固定化した抗体や酵素等に反応する抗原などが含まれていれば、図１４に示すようにその抗体や酵素等を固定化した部分２６でＳＰＲ角度の変化が生じ、他の部分２５とは異なって見える。このように、被測定物質中の成分をそれぞれの抗体や酵素等に反応する物質について多成分分析することができる。

【００２８】また、図１５に示すように、すべてに同じ抗体２３などを固定化しておき、異なる被測定物質２４を加えれば、多種類の被測定物質中の１成分の検出が可能である。

【００２９】さらに、2次元イメージング表面プラズモン共鳴測定装置と、分割した金属薄膜を用いて、遺伝子の測定を行うことができる。

【００３０】異なる配列を持つＤＮＡの末端にチオール基等を修飾し、金属薄膜上に固定化する。被測定物質としてさまざまな化学物質を使えば、特定のＤＮＡに与える影響を屈折率変化として捉えることができる。また、配列のわかっているＤＮＡを金属薄膜上に固定化し、未知の配列を持つＤＮＡを被測定物質とすれば、屈折率変化の生じた金属薄膜の座標から未知の遺伝子の塩基配列を知ることができる。

【００３１】本発明ではさらに、本装置をイメージセンサとして用いることができる測定方法を確立した。金属薄膜上に単に、屈折率分布をもった物体を置いた場合でもそのままで屈折率分布やその形状を測定できるばかりでなく、図１６のように、金属薄膜２１上に生物や細胞などが取りこんだり排出する物質に感応する抗体や酵素等を固定化しセンサ膜を作製する。その上に生物や細胞２７を配置する。すると、代謝により生物や細胞の周辺のＳＰＲに変化が生じ、代謝の様子２８や代謝しながら移動した跡２９を図１７のように測定することができるというものである。

【００３２】

【実施例】以下、本発明を実施例によりさらに具体的に説明するが、本発明はこれら実施例に限定されない。

実施例1

図5に示したような構造を持つ装置を作製し、測定を行った。光源はＬＥＤとし、金属薄膜はプリズムと同じ屈折率のＢＫ７のガラス板にスパッタ法により形成した。金属薄膜の区切りは、金属薄膜上に光感応性高分子を均一に塗布し、マスクを通して紫外線を照射することによって必要な部分を硬化させ、未硬化部分は有機溶媒で落として作製した。そのパターンは図１２に示すような格子状のパターンである。金属薄膜をスパッタしたガラス板をマッチングオイルでプリズムに貼り付けて、被測定物質を水として測定を行った。図１８に示すように、アスペクト比未変換のＣＣＤの生の画像は、左右方向に圧縮された画像となっているが、コンピュータで画像処理を施し、図１９に示すような元の金属薄膜のパターンと同じアスペクト比を持つ画像が得られた。ここでは、水で表面プラズモン共鳴が起こるような入射角に固定しているので、高分子のパターンが無い部分（金属薄膜に直接、水が接している部分）は暗く映っている。

【００３３】実施例２

図6に示すような構造を持つ装置を作製し、測定を行った。光源はＬＥＤの光をマルチモード光ファイバに入射し、ファイバの中を伝送され、出射された光を用いた。アスペクト比変換部分にはプリズムを２つ使用したもので、各々のプリズムはモータで回転が可能で、アスペクト比は自由に変えることができる。入射角の変化によるアスペクト比変換の比率は自動で変わるようになっている。金属薄膜は実施例１と同じ方法で作製したものを用いて、被測定物質を水として測定を行った。測定結果は図２０に示すように、元の金属薄膜のパターンと同じアスペクト比を持つ画像が得られた。

【００３４】実施例３

実施例２の装置を使用して、金属薄膜は実施例１と同じ方法で作製したものを使用し、被測定物質を水とし、入射角度を走査しながら測定を行った。角度の変化により、アスペクト比変換機構の比率が自動で変化し、入射角度を変化させてもアスペクト比が金属薄膜と同じ画像が得られた。画像を観察すると、全反射、水によるＳＰＲの吸収、全反射へと変化する様子が見られた。また、画像データから任意の点を指定し、その点での受光光量の変化を抽出し、その変化をグラフに表すことも可能である。

【００３５】実施例４

実施例1と同じ方法で４つの領域（ａ、ｂ、ｃ、ｄ）に区切った金の薄膜パターンを作製した。各金属膜はアビジンで修飾した。５’の位置をビオチンで修飾したＤＮＡのＴＥＳ緩衝溶液をマイクロディスペンサーで滴下し、ビオチン－アビジン結合によりＤＮＡプローブを金属膜に固定化した。ここで、領域ａにはビオチン－５’－ＧＧＣＡＧＴＧＣＣＴＣＡＣＡＡ－３’、領域ｂにはビオチン－５’－ＧＧＣＡＧＴＧＧＣＴＣＡＣＡＡ－３’、領域ｃにはビオチン－５’－ＧＧＣＡＧＴＧＡＣＴＣＡＣＡＡ－３’、領域ｄにはビオチン－５’－ＧＧＣＡＧＴＧＴＣＴＣＡＣＡＡ－３’のＤＮＡを固定化した。非特異的に吸着したＤＮＡはＮａＯＨ溶液で洗浄した。作製したセンサーをＳＰＲ用のフローセルに組み込んだ。

【００３６】次にターゲットＮｏ．１として５’－ＴＴＧＴＧＡＧＧＣＡＣＴＧＣＣ－３’のＨＥＰＥＳ緩衝溶液をセンサに流しながらＳＰＲ測定を行ったところ、領域ａの部分のみＳＰＲシグナルの変化を観測することが

できた。これはターゲットＮｏ．１が領域ａに固定化されたＤＮＡと相補関係にあるために、ハイブリダイゼーションを起こしたためである。次に高濃度の尿素を流したところ、領域ａの部分のＳＰＲシグナルは他の領域と同じになった。尿素によって二重らせんがほどけセンサを再生することができた。同様にターゲットＮｏ．２として５’－ＴＴＧＴＧＡＧＣＣＡＣＴＧＣＣ－３’のＨＥＰＥＳ緩衝溶液を流したところ、領域ｂの部分のみＳＰＲシグナルの変化を観測することができた。

【００３７】以上示したように、インターカレータや蛍光ラベルを使用することなくＤＮＡを選択的に検出することができた。金薄膜をさらに多くの領域にわけ、異なるＤＮＡを配置すれば、より多くの塩基の解析が可能になる。これにはフォトリソグラフィを応用した金薄膜上へのＤＮＡの直接合成が利用できる。

【００３８】実施例５

実施例１と同じ方法で５つの領域（ａ、ｂ、ｃ、ｄ、ｅ）に区切った金の薄膜パターンを作製した。領域ａには西洋わさびペルオキシダーゼ（ＨＲＰ）を含んだＯｓポリマー（ＢＡＳ社製）を塗布し、その後ヒスタミン酸化酵素を固定化した。領域ｂには同じくＨＲＰを含んだＯｓポリマーを塗布し、その後グルタミン酸酸化酵素を固定化した。領域ｃにはＨＲＰを含んだＯｓポリマーを塗布し、その後、ラクテート（乳酸）酸化酵素を固定化した。領域ｄ、ｅには参照計測として、それぞれ、Ｏｓポリマーのみと、何も塗布しない領域を設けた。

【００３９】ヒスタミン酸化酵素はヒスタミンを分解し、過酸化水素を生成する。過酸化水素はＨＲＰによって還元され水となり、ＨＲＰ自身は酸化状態となり、Ｏｓの価数が２価から３価となる。グルタミン酸酸化酵素はグルタミン酸を、ラクテート酸化酵素はラクテートを分解し過酸化水素を生成し、同様の反応を起こす。

【００４０】したがって、試料としてヒスタミンを加えれば領域ａの明暗が変化し、グルタミン酸を加えれば領域ｂの明暗が変化し、ラクテートを加えれば領域ｃの明暗が変化する。試料として過酸化水素が含まれている場合、領域ａ、ｂ、ｃともに変化してしまうが、領域ｄも明暗が変化するので検出が可能である。その変化する領域とその領域の受光光量から試料中のヒスタミン、グルタミン酸、ラクテート、過酸化水素の検出、定量をすることができる。また、高濃度の試料が流れてきても、領域ａ、ｂ、ｃ、ｄの明暗の反応領域と、領域ｅの受光光量変化から検出・定量することが可能である。

【００４１】以上の方法で、金属薄膜の区切りをより多くし、固定化する酵素をより多くすることによって、より多種類の同時検出、定量が可能である。

【００４２】実施例６

実施例２で作製した装置を用いて、ガラス板に金薄膜をスパッタしたものを計測チップとし、計測チップをプリズムの上にマッチングオイルで貼り付ける。そして、計測チップの上に細胞を置く。すると、屈折率の違いから細胞の像を得ることが可能である。さらに、細胞の代謝にかかわる物質に特異的に反応する抗体や酵素等を固定化しておけば、細胞の代謝の様子も観察できる。

【００４３】

【発明の効果】以上説明したように、本発明に係る２次元イメージング表面プラズモン共鳴測定装置及び測定方法によれば、ＳＰＲ測定時の反射光のアスペクト比の歪みを適性に補正できるようにしたので、被測定物質の実際のアスペクト比に合致した測定データを正確に得ることができる。

【００４４】また、金属薄膜を適切に区切って２次元ＳＰＲ測定に供することにより、抗原抗体反応や遺伝子、生物や細胞の物質代謝などの様子を多チャンネルで測定することができる。

【図面の簡単な説明】

【図１】従来のＳＰＲセンサの概略構成図である。

【図２】従来のＳＰＲセンサで得られる測定結果の一例を示す特性図である。

【図３】従来の２次元ＳＰＲセンサの概略構成図である。

【図４】（ａ）は２次元ＳＰＲセンサに使用される金属薄膜パターンであり、（ｂ）は上記パターンを従来の２次元ＳＰＲセンサで測定した場合の測定結果の概略図である。

【図５】本発明に係るアスペクト比をコンピュータで補正するシステムの概略構成図である。

【図６】アスペクト比を光学的手段で補正する場合のシステムの概略構成図である。

【図７】アスペクト比を機械的手段で補正する場合のシステムの概略構成図である。

【図８】図７のシステムの角度を変えた場合の概略構成図である。

【図９】本発明に係る別の形態を示す概略構成図である。

【図１０】本発明に係るさらに別の形態を示す概略構成図である。

【図１１】本発明に係るさらに別の形態を示す概略構成図である。

【図１２】２次元ＳＰＲセンサに使用される金属薄膜パターンの概略図である。

【図１３】異なる抗体や酵素等を固定化した多チャンネルセンサとしての応用を示す概略断面図である。

【図１４】多チャンネルセンサの測定結果の一例を示す概略図である。

【図１５】異なる試料を測定する多チャンネルセンサとしての応用を示す概略断面図である。

【図１６】イメージセンサとしての応用の一例を示す概略図である。

【図１７】図１６のイメージセンサとして応用した時の

測定結果の概略図である。

【図１８】実施例１におけるアスペクト比変換機構なしの場合の測定結果を撮影した図である。

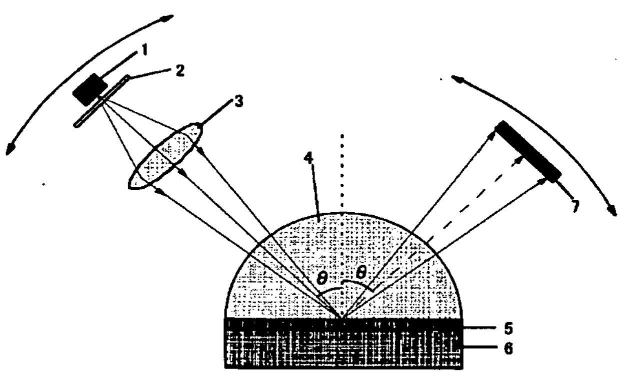
【図１９】実施例１におけるコンピュータにてアスペクト比変換を行った場合の測定結果を撮影した図である。

【図２０】実施例２におけるアスペクト比変換を行った場合の測定結果を撮影した図である。

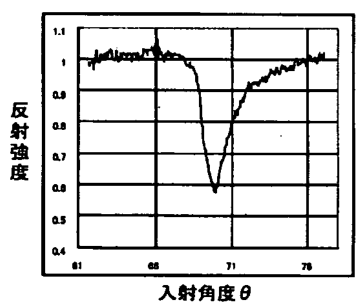
【符号の説明】

1　光源
2　偏光板・偏光子
3　レンズ
4　プリズム
5　金属薄膜
5ａ　計測面を形成する、複数に区切られた金属薄膜
5ｂ　観測されるデータ取得面
6　試料（被測定物質）
7　光電子検出器
8　セル
9　ＣＣＤカメラ
１０　コンピュータ
１１　アスペクト比補正機構
１２　実際の計測面
１３　観測される歪んだ画像
１４　補正された画像
１５　入射光側アーム
１６　出射光側アーム
１７　アーム
１８　ジョイント部
１９　アーム
２０　透明体
２１　分割された金属薄膜
２２　高分子
２３　抗体や酵素等
２４　被測定物質
２５　抗体や酵素等を固定化していない部分
２６　抗体や酵素等を固定化した部分
２７　生物や細胞
２８　生物や細胞などが代謝している部分
２９　代謝跡
３１、４１　熱的光源
３２、３５　偏光子
３３、４２、５３　プリズム
３４、４３　金属薄膜
３６、５５　ＣＣＤカメラ
４４　２次元画像分光システム
５１　半導体レーザ光源
５２　格子状のレーザビーム
５４　マトリックス状に区画された金属薄膜

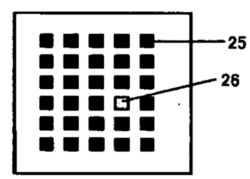
【図１】



【図２】



【図１４】



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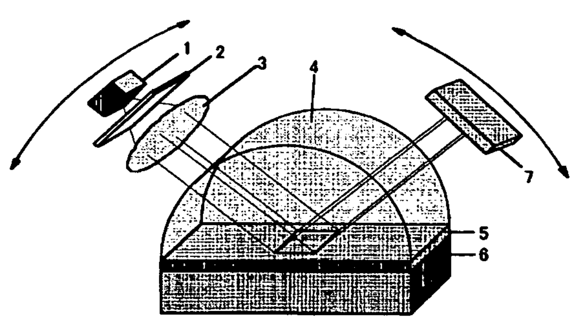

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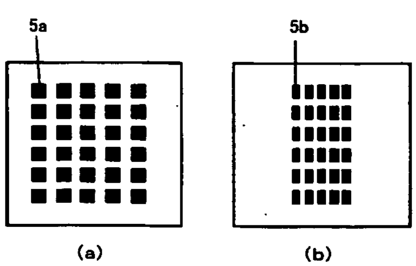

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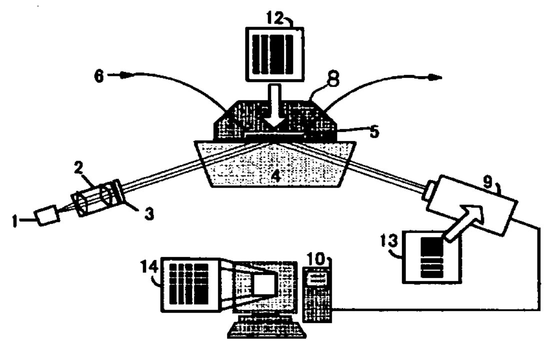

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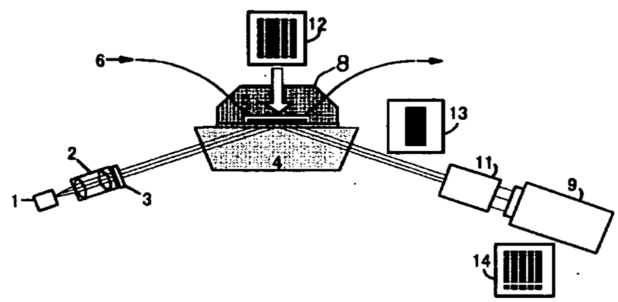

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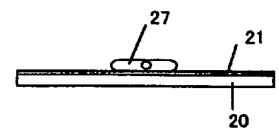

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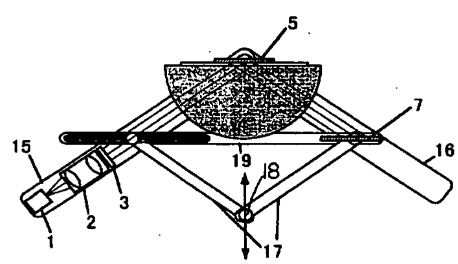

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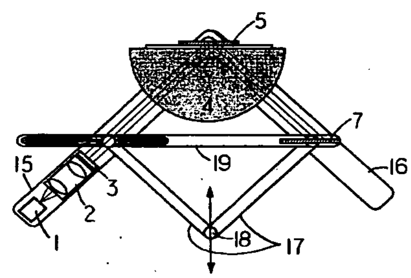

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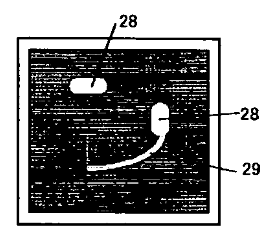

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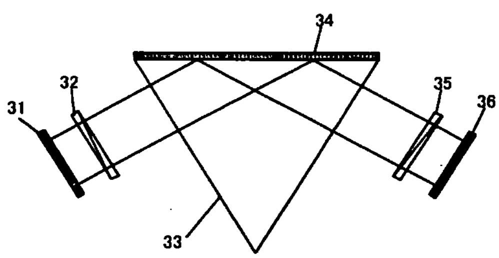

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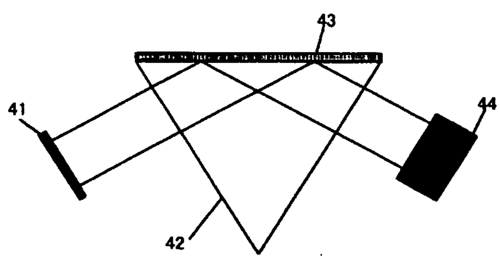

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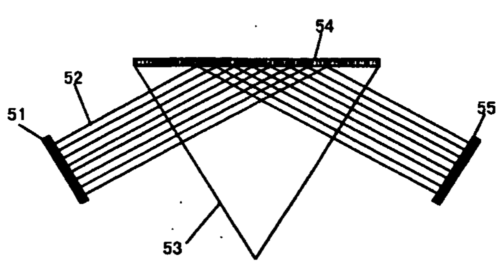

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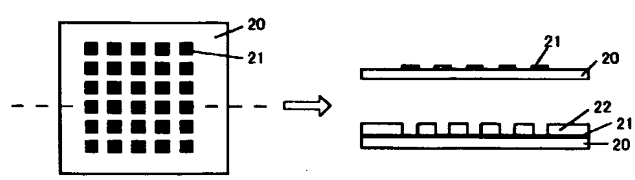

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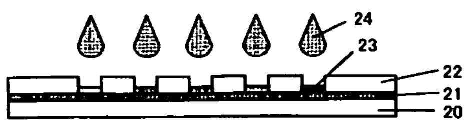

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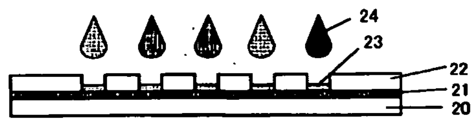

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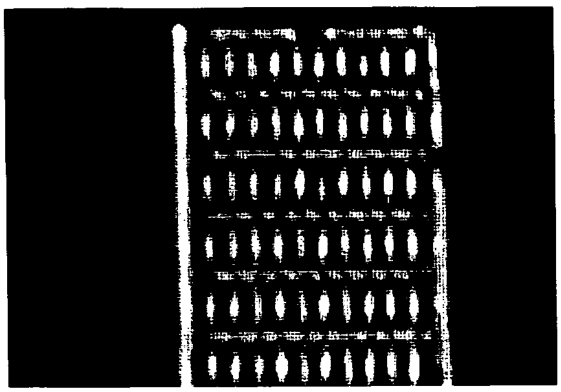

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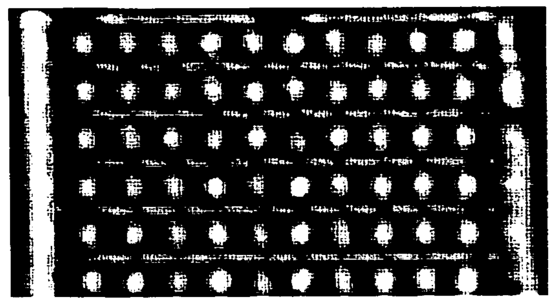

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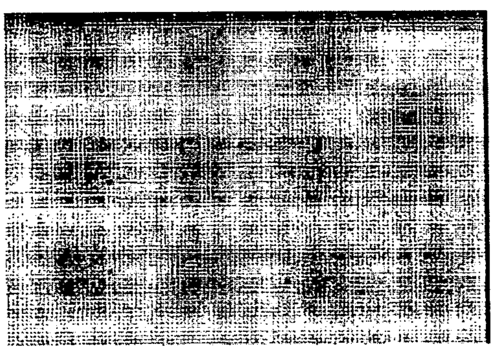

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Ｆターム(参考)　2G045 AA40 CB30 DA12 DA13 DA37
DA80 FA07 FA12 FB01 FB02
FB03 GC11 JA01 JA07
2G059 AA01 BB04 BB12 CC16 DD03
DD13 EE02 EE05 EE12 FF01
FF11 GG01 GG04 HH02 JJ11
JJ12 JJ17 JJ19 KK04 MM01
PP04

* NOTICES *

CLAIMS

[Claim(s)]
[Claim 1] The prism equipped with the metal thin film which touches the quality of a device under test on the front face, and the ON light means to which incidence of the light from the light source is carried out from a prism side as the parallel flux of light of p-polarized light light, It has a light-receiving means to measure the two-dimensional light-receiving quantity of light of the reflected light including absorption by the surface plasmon resonance phenomenon from a metal thin film. The two-dimensional imaging surface plasmon resonance measuring device characterized by having a means to amend the distortion to the aspect ratio in the contact surface to the metal thin film of the quality of a device under test of the aspect ratio of the reflected light by carrying out incidence of the flux of light aslant to the field of said metal thin film with said ON light means.
[Claim 2] The measuring device according to claim 1 with which a means to amend said aspect ratio consists of data-processing means, such as a computer.
[Claim 3] The measuring device according to claim 1 with which a means to amend said aspect ratio consists of a means to amend the reflected light using an anamorphic lens.
[Claim 4] The measuring device according to claim 1 with which a means to amend said aspect ratio consists of a means to amend the reflected light using the prism for aspect ratio amendment.
[Claim 5] The measuring device according to claim 1 which consists of a means to have the device to which it is made to move according to the include angle of the reflected light while a means to amend said aspect ratio had kept parallel to a measuring plane the component which measures the two-dimensional light-receiving quantity of light.
[Claim 6] The measuring device according to claim 1 to 5 with which a

means to amend said aspect ratio consists of a means by which the amount of amendments of an aspect ratio is changeable into arbitration.
[Claim 7] The measuring device according to claim 1 to 6 with which the light spread with the optical fiber is used for the light source.
[Claim 8] The measuring device according to claim 1 to 7 which specifies a certain one point or two or more points, and has processing units, such as a computer which can draw the reflectivity in the point as an extract and a graph, in two or more image data which scanned the include angle and was obtained.
[Claim 9] The measuring device according to claim 1 to 8 which has the device which can move both the quality of a device under test, or prism and the quality of a device under test in parallel with a measuring plane in order to measure the field of the arbitration of the quality of a device under test.
[Claim 10] A two-dimensional imaging surface plasmon resonance measuring device according to claim 1 to 9 is used. By producing the sensor film which fixed an antibody which is different on a break and each divided metal thin film in a metal thin film, the enzyme, etc., and contacting the same quality of a device under test on the sensor film Or the two-dimensional imaging surface plasmon resonance measuring method characterized by performing the quantum and the quality of the chemical of varieties to coincidence by producing the sensor film which fixed same antibody, same enzyme, etc. as each divided metal thin film, and contacting quality of a device under test which is different in each.
[Claim 11] The measuring method of claim 10 which detects the chemical which affects a gene by producing the sensor film which fixed the gene and introducing various kinds of chemicals as quality of a device under test.
[Claim 12] The measuring method of claim 10 which detects the array of a strange gene from the coordinate in the metal thin film front face from which the reaction was started by producing the sensor film which fixed the gene with a known array, and introducing a gene with an array strange as quality of a device under test.
[Claim 13] The measuring method according to claim 10 which detects the chemical which affects a biological substance by producing the sensor film which fixed the biological substance and introducing various kinds of chemicals as quality of a device under test.
[Claim 14] The measuring method according to claim 10 to 13 which measures refractive-index distribution of the quality of a device under test on a metal thin film.

[Claim 15] The two-dimensional imaging surface plasmon resonance measuring method characterized by measuring the situation of the metabolic turnover by producing the sensor film which arranges or fixed the antibody which induces the matter which a living thing and a cell take in on a metal thin film, or the matter to discharge, the enzyme, etc., and placing a living thing and a cell on it using a two-dimensional imaging surface plasmon resonance measuring device according to claim 1 to 9.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates the special material in a measured solution to a quantum or the surface plasmon resonance measuring device measured qualitatively, and a measuring method using optical system. Refractive-index change of quality of a device under test, such as a liquid and gas, is detected in detail using the surface plasmon resonance phenomenon in the metal thin film which touched the quality of a device under test, quality and quantum measurement are performed, and it is related with the measuring device which enables two-dimensional measurement of a metal thin film side, and its measuring method.
[0002]
[Description of the Prior Art] Conventionally, measurement which used color reaction and an immunoreaction by chemical process measurement, environmental measurement, a clinical laboratory test, etc. is performed. However, it is necessary to carry out the sample extract of the quality of a device under test and also, and there are problems, such as needing complicated actuation and a complicated marker, by such measuring method. On the other hand, the sensor which used the optical-pumping surface plasmon resonance phenomenon by high sensitivity as a possible sensor of the quality and quantum measurement of the chemical in the quality of a device under test is proposed and put in practical use, without needing a marker. Hereafter, surface plasmon resonance (Surface Plasmon Resonance) may be abbreviated to SPR, and may be used.
[0003] As a system which constitutes a SPR sensor, as shown, for example in drawing 1

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[0003] As a system which constitutes a SPR sensor, as shown, for example in drawing 1 Incidence of the polarizing plate 2 which lets only

p-polarized light light pass for the light emitted from the light source 1, and the condenser lens 3 is carried out with the incident angle in the through quantity refractive-index prism 4. The system which irradiates the metal thin film 5 with the sensor film which touches the quality 6 of a device under test, and detects a change of the reflected light from the metal thin film 5 on the strength with the photoelectron detector 7 through prism 4 is common.
[0004] The light emitted from the light source 1 makes an evanescent wave occur in the interface of prism 4 and the metal thin film 5, and the wave number is defined by the degree type.
kev=kp np sintheta -- here -- kp The wave number of incident light, and np The refractive index of prism and theta are incident angles.
[0005] On the other hand, a surface plasmon wave arises and the wave number is defined by the front face of the metal thin film 5 by the degree type.
ksp=(c/omega) -root (epsilonn2/(epsilon+n 2))
Here, for c, the velocity of light and omega are [the dielectric constant of a metal thin film and n of angular frequency and epsilon] the refractive indexes of the quality of a device under test.
[0006] When it is the angle of incidence theta whose wave number of this evanescent wave and a surface plasmon wave corresponds, as an evanescent wave is used for excitation of surface plasmon, for example, it is shown in drawing 2 , the quantity of light observed as the reflected light decreases.
[0007] In order to depend for a SPR phenomenon on the refractive index of the quality of a device under test which touched prism and a metal thin film For example, when quality of a device under test is used as water, it can detect as a curve which has the minimum at a certain fixed include angle as shown in drawing 2 . The quantum of special material can be performed by measuring refractive-index change of the antibody combined with the antigen by fixing an antibody etc. on about [measuring the refractive-index change by chemical concentration change of the quality of a device under test etc.], and a metal thin film.
[0008] However, the correspondence to measurement of refractive-index distribution of the quality of a device under test and measurement of many samples is demanded in recent years.
[0009] Then, in order to measure refractive-index distribution of the quality of a device under test, as shown in drawing 3 , the light by which through incidence was carried out [light / from the light source 1] in the polarizing plate 2 is changed into parallel light with a lens 3, and the

equipment which measures the SPR phenomenon of the quality 6 of a device under test on the metal thin film 5 which had a field through prism 4 with the CCD (charge-coupled device) camera 7 is proposed. Of course, since parallel light has only one incident angle, the device in which an incident angle and angle of reflection may always be driven is required for it. However, with the equipment of a system like drawing 3 , in order to observe the light reflected with a certain include angle, there was a problem that aspect ratios (aspect ratio) differed, by the actual measuring plane, for example, the measurement side where two-dimensional array of the metal thin film 5a (for example, golden thin film) was carried out as shown in drawing 4 (a), and data acquisition side 5b (drawing 4 (b)) observed.
[0010] Moreover, although the equipment which measures some samples to coincidence by arranging incident light in juxtaposition and similarly arranging the cel for paying a sample in juxtaposition about the correspondence to measurement of many samples is known, the coincidence measurement beyond it is impossible and has a limitation.
[0011]
[Problem(s) to be Solved by the Invention] This invention is made in view of such the present condition, the technical problem is characterized by having the function which amends an aspect ratio, and it is in offering the two-dimensional imaging surface plasmon resonance measuring device with which the measurement data corresponding to the actual aspect ratio of the quality of a device under test is obtained. Furthermore, another technical problem of this invention is to offer the approach of measuring situations, such as multi-channel measurement of biological substances, such as an antibody antigen reaction which used the equipment by this invention, or a gene, a living thing, and a metabolic turnover of the matter of a cell.
[0012]
[Means for Solving the Problem] In order to solve the above-mentioned technical problem, this invention adds an aspect ratio amendment function so that the image obtained in a two-dimensional imaging surface plasmon resonance measuring device may be obtained similarly to the aspect ratio of the quality of a device under test. Namely, the two-dimensional imaging surface plasmon resonance measuring device concerning this invention The prism equipped with the metal thin film which touches the quality of a device under test on the front face, and the optical means to which incidence of the light from the light source is carried out from a prism side as the parallel flux of light of p-polarized

light light, It has a light-receiving means to measure the two-dimensional light-receiving quantity of light of the reflected light including absorption by the surface plasmon resonance phenomenon from a metal thin film. It is characterized by having a means to amend the distortion to the aspect ratio in the contact surface to the metal thin film of the quality of a device under test of the aspect ratio of the reflected light by carrying out incidence of the flux of light aslant to the field of said metal thin film with said ON light means.

[0013] In aspect ratio amendment, the operation approach which amends the data detected with the photodetector of a light-receiving means using arithmetic units, such as a computer, the thing which amends with an optical means before the reflected light goes into a photodetector, and the mechanical approach made to drive so that the location of the photodetector itself can always be amended can be considered.

[0014] By amendment of such an aspect ratio, the image of the same aspect ratio as the quality of a device under test is obtained, and it becomes applicable as two-dimensional image sensors.

[0015] The two-dimensional imaging surface plasmon resonance measuring method concerning this invention By fixing a metal thin film for a break, an antibody, an enzyme which divide and are different on each metal thin film, etc., producing the sensor film using the above two-dimensional imaging surface plasmon resonance measuring devices, and contacting the same quality of a device under test on the sensor film Or it consists of an approach characterized by performing the quantum and the quality of the chemical of varieties to coincidence by producing the sensor film which fixed same antibody, same enzyme, etc. as each divided metal thin film, and contacting quality of a device under test which is different in each. That is, a break is formed in a metal thin film so that it can be used as a multi-channel sensor, and it enables it to put a sample which fixes a different antibody, a different enzyme, etc. or is different on the metal thin film divided into each.

[0016]

[Embodiment of the Invention] Below, the gestalt of each operation of this invention is explained, referring to a drawing. The first description of this invention is the point which gave the aspect amendment function. As an aspect ratio amendment device by the computing type, the example is shown in drawing 5 . The aspect ratio which included SPR absorption from the metal thin film 5 once detects the reflected light which is not normal with CCD camera 9 etc., and amends the detected image to a suitable aspect ratio with processing units, such as a

computer 10. Although the data observed with CCD camera 9 are distorted like an image 13 to the actual measurement side 12 of the sample 6 (quality of a device under test) in a cel 8, an aspect ratio is amended by computer 10 by the same image 14 as the actual measurement side 12. However, by this approach, an image may become coarse with the pixel consistency of CCD at the time of amendment, and complement processing of a suitable image may be needed.
[0017] As an aspect ratio amendment device by the optical means, a cylindrical lens or the anamorphic lens which combined it, the system which combined prism can be used. The example of the system is shown in drawing 6 . The same image 14 as a sample with an actual aspect ratio can be obtained by detecting with CCD camera 9 etc. through the aspect ratio amendment device which used the anamorphic lens for the flux of light which is not normal as for the aspect ratio reflected from the metal thin film 5, or the aspect ratio amendment device 11 which carried out combination use of equipment of the prism for amendment.
[0018] In the above two systems, since the aspect ratio of the reflected light changes with whenever [incident angle], it is necessary for the correction value to be freely changeable.
[0019] As an approach of proofreading an aspect ratio by the mechanical approach, a device as shown in drawing 7 and drawing 8 can be used. In drawing, the light source 1, the lens 2, and the polarizer 3 are attached in the arm 15 by the incident light side. The arm 16 is attached in the outgoing radiation light side, the auxiliary device in which two arms 17 turn into both the arms 15 and 16 from the link mechanism connected in the joint section 18 is attached, and an arm 16 always drives along with outgoing radiation light to incident light. And an arm 19 can be attached further. This arm 19 is always parallel to a metal thin film side, and has attached the two-dimensional photo detector 7 (electric eye) in the outgoing radiation light side of an arm 19. The joint part of each part is movable, as for the joint of an arm 19 and an arm 15, the slit has opened on the arm 19, and it can slide. The joint section 18 of an arm 17 moves on a perpendicular guide to a sample. According to this approach, as shown in drawing 7 and drawing 8 , even if it changes the include angle of incident light, it turns out that a photo detector 7 always becomes parallel to a metal thin film side, and include-angle change of the outgoing radiation light accompanying change of whenever [incident angle] is also followed. The image which had an aspect ratio normal have [no special aspect ratio translator] always on this photo detector 7 can be obtained.

[0020] In this invention, an optical fiber can be used as the light source. In the mere LED light source and mere LD light source, the profile of the outgoing radiation quantity of light was not uniform, and when carrying out incidence to a metal thin film, brightness had the fault that spots will be made. By letting light pass to an optical fiber once, the profile of the to some extent uniform outgoing radiation quantity of light was obtained, and it became possible to apply light to a metal thin film side as the uniform flux of light. Moreover, in order to obtain parallel light without an angle error, the point light source is required, but if the optical outgoing radiation edge of an optical fiber is chosen as the light source, according to the purpose, a core diameter can be chosen from several micrometers to hundreds of micrometers.
[0021] Moreover, in this invention, a device as shown in drawing 9 - drawing 11 can also be added. In drawing 9 , the reflected light from the metal thin film 34 which let [the parallel light from the thermal light source 31 which carries out outgoing radiation of the monochrome parallel light, for example] through and prism 33 pass for the polarizer 32 also lets a polarizer 35 pass, and obtains an image with CCD camera 36. It becomes possible by combining polarizers 32 and 35 to raise resolution.
[0022] It enables it to have seen spectrum change by using the white light in drawing 10 using the thermal light source 41 which carries out outgoing radiation of the white parallel light. prism 42 -- letting it pass -- the reflected light from the metal thin film 43 -- a two-dimensional image -- a spectrum -- spectrum change of the data acquired can be seen by observing combining a system 44, a imaging spectroscope, and a piezo drive.
[0023] In drawing 11 , outgoing radiation of the grid-like laser beam 52 is carried out, for example from the semiconductor laser light source 51, and the reflected light from the metal thin film 54 has been obtained as an image with CCD camera 55 through prism 53. The coincidence measurement of many channels becomes possible by applying the grid-like laser beam 52 separately to each metal thin film 54 divided in the shape of a matrix.
[0024] This invention offers the measuring method as a multichannel sensor using the above two-dimensional imaging surface plasmon resonance measuring devices. The approach fixes an antibody, an enzyme, etc. which induce matter which divided for example, the metal thin film upwards, and is different, respectively, and can carry out [quality and] a quantum in the matter of varieties at once. Or an

antibody, an enzyme, etc. same on the divided metal thin film are fixed, different quality of a device under test is added to each, and quality and a quantum are performed.
[0025] Although it is having structure as shown in drawing 12 and the metal thin film used for measurement can be constituted in what the divided metal thin film 21 arranges respectively independently on the transparent body 20, the structure which divides the metal thin film 21 with a macromolecule 22 etc., the configuration, especially the quality of the material, etc. are not limited.
[0026] (3) which produces a pattern with macromolecules, such as light-sensitive nature resin, on (2) metal thin film which uses a mask when the approach of division of a metal thin film attaches (1) metal thin film, although approaches, such as sticking the macromolecule sheet which the pattern attached beforehand, can be considered It is more desirable for a break to be between metal thin films, if an antibody, an enzyme, etc. which are different in each exposed metal thin film are fixed or it considers to introduce different quality of a device under test.
[0027] If the antigen reacted to the antibody fixed in the sample by an antibody, an enzyme, etc. which are different as shown in the divided metal thin film which was produced as mentioned above at drawing 13 fixing 23, and adding the quality 24 of a device under test further, an enzyme, etc. is contained, as shown in drawing 14 , change of a SPR include angle arises in the part 26 which fixed the antibody, enzyme, etc., and it differs and is visible in other parts 25. Thus, the multicomponent analysis of the component in the quality of a device under test can be carried out to each antibody, enzyme, etc. about reactant.
[0028] Moreover, if quality 24 of a device under test which fixes the same antibody 23 as all etc. and is different is added as shown in drawing 15 , detection of one component in the quality of a device under test of varieties is possible.
[0029] Furthermore, a gene can be measured using a two-dimensional imaging surface plasmon resonance measuring device and the divided metal thin film.
[0030] A thiol group etc. is embellished at the end of DNA with a different array, and it fixes on a metal thin film. If various chemicals as quality of a device under test are used, the effect which it has on specific DNA can be regarded as refractive-index change. Moreover, DNA which the array understands can be fixed on a metal thin film, and the base sequence of a strange gene can be known from the coordinate

of the metal thin film with which the quality of a device under test, then refractive-index change produced DNA with a strange array.
[0031] In this invention, the measuring method which can use this equipment as image sensors was established further. On a metal thin film, like drawing 16 , even when the body which only had refractive-index distribution is placed, it remains as it is, and a living thing, a cell, etc. take in on the metal thin film 21, or it fixes an antibody, an enzyme, etc. which induce the matter to discharge, and it not only can measure refractive-index distribution and its configuration, but produces the sensor film. A living thing and a cell 27 are arranged on it. Then, change arises in surrounding SPR of a living thing or a cell by the metabolic turnover, and a metabolic situation 28 and the metabolic marks 29 where it moved while being metabolized can be measured like drawing 17 .
[0032]
[Example] Hereafter, although an example explains this invention still more concretely, this invention is not limited to these examples.
It measured by producing equipment with structure as shown in example 1 drawing 5 . The light source was set to LED and formed the metal thin film in the glass plate of BK7 of the same refractive index as prism by the spatter. When the break of a metal thin film applied a light-sensitive nature macromolecule to homogeneity on a metal thin film and irradiated ultraviolet rays through a mask, the required part was stiffened, and a part for a non-hard spot was dropped on the organic solvent, and was produced. The pattern is a pattern of the shape of a grid as shown in drawing 12 . The glass plate which carried out the spatter of the metal thin film was stuck on prism in matching oil, and it measured by using quality of a device under test as water. As shown in drawing 18 , although the raw image of aspect ratio unconverted CCD had turned into an image compressed into the longitudinal direction, the image processing was performed by computer and the image with the same aspect ratio as the pattern of the original metal thin film as shown in drawing 19 was obtained. Here, since it is fixing to an incident angle from which surface plasmon resonance takes place with water, the part (part to which water is in contact with the metal thin film directly) without the pattern of a macromolecule has been reflected darkly.
[0033] It measured by producing equipment with structure as shown in example 2 drawing 6 . The light source carried out incidence of the light of LED to the multimode optical fiber, and used the light by which outgoing radiation was transmitted and carried out in the inside of a fiber. It is what used two prism for the aspect ratio conversion part, and

each prism can be rotated by the motor and an aspect ratio can be changed freely. The ratio of the aspect ratio conversion by change of an incident angle changes automatically. The metal thin film measured by using quality of a device under test as water using what was produced by the same approach as an example 1. The image in which a measurement result has the same aspect ratio as the pattern of the original metal thin film as shown in drawing 20 was obtained.
[0034] Using the equipment of example 3 example 2, what was produced by the same approach as an example 1 was used, quality of a device under test was used as water, and the metal thin film measured, scanning whenever [incident angle]. Even if the ratio of an aspect ratio translator changes automatically and changed whenever [incident angle] by change of an include angle, the image as a metal thin film with the same aspect ratio was obtained. Observation of the image looked at signs that it changed to the absorption of SPR by total reflection and water, and total reflection. Moreover, it is also possible to specify the point of arbitration from image data, to extract change of the light-receiving quantity of light in the point, and to express the change with a graph.
[0035] The thin film pattern of the gold divided into four fields (a, b, c, d) by the same approach as example 4 example 1 was produced. Each metal membrane was embellished with avidin. The TES buffer solution of DNA which embellished the location of 5' with the biotin was dropped with the micro dispenser, and the DNA probe was fixed in the metal membrane by biotin-avidin association. Here, in Field a, DNA of biotin-5'-GGCAGTGTCTCACAA-3' was fixed to biotin-5'-GGCAGTGACTCACAA-3' and Field d in biotin-5'-GGCAGTGGCTCACAA-3' and Field c at biotin-5'-GGCAGTGCCTCACAA-3' and Field b. The NaOH solution washed DNA to which it stuck nonspecific. The produced sensor was built into the flow cell for SPR.
[0036] Next, when SPR measurement was performed for the HEPES buffer solution of 5'-TTGTGAGGCACTGCC-3' in the sensor with the sink as target No.1, only the part of Field a was able to observe change of a SPR signal. Since target No.1 has DNA and complementary relation which were fixed by Field a, this is because hybridization was started. Next, when the high-concentration urea was poured, the SPR signal of the part of Field a became the same as other fields. With the urea, the double helix was able to come loose and the sensor was able to be reproduced. When the HEPES buffer solution of 5'-

TTGTGAGCCACTGCC-3' was similarly poured as target No.2, only the part of Field b was able to observe change of a SPR signal.
[0037] DNA was able to be detected alternatively, without using an intercalator and a fluorescence label, as shown above. If a golden thin film is divided into much more fields and different DNA is arranged, the analysis of more bases will be attained. Direct composition of DNA to the golden thin film top adapting a photolithography can be used for this.

[0038] The thin film pattern of the gold divided into five fields (a, b, c, d, e) by the same approach as example 5 example 1 was produced. Os polymer (product made from BAS) containing horseradish peroxidase (HRP) was applied to Field a, and histamine oxidizing enzyme was fixed after that. Os polymer which contained HRP as well as Field b was applied, and glutamic-acid oxidizing enzyme was fixed after that. Os polymer containing HRP was applied to Field c, and lactate (lactic acid) oxidizing enzyme was fixed after that. Os polymer and the field which nothing applies were established in Fields d and e as reference measurement, respectively.
[0039] Histamine oxidizing enzyme disassembles a histamine and generates a hydrogen peroxide. It is returned by HRP and a hydrogen peroxide serves as water, the HRP itself will be in an oxidation state and the valence of Os serves as trivalent from divalent. Glutamic-acid oxidizing enzyme decomposes glutamic acid, lactate oxidizing enzyme decomposes lactate, a hydrogen peroxide is generated, and the same reaction is caused.
[0040] Therefore, if a histamine is added as a sample, the light and darkness of Field a will change, if glutamic acid is added, the light and darkness of Field b will change, and if lactate is added, the light and darkness of Field c will change. When the hydrogen peroxide is contained as a sample, Fields a, b, and c will change, but since light and darkness change, Field d is detectable. Detection of the histamine in a sample, glutamic acid, lactate, and a hydrogen peroxide and a quantum can be carried out from the light-receiving quantity of light of the changing field and its field. Moreover, even if a high-concentration sample flows, it is possible detection and to carry out [light-receiving quantity of light change of the reaction field of the light and darkness of Fields a, b, c, and d and Field e to] a quantum.
[0041] By the above approach, the break of a metal thin film is made [more], and coincidence detection of varieties and a quantum are more possible by making [more] the enzyme to fix.

[0042] What carried out the spatter of the golden thin film to the glass plate is considered as a measurement chip using the equipment produced in the example 6 example 2, and a measurement chip is stuck in matching oil on prism. And a cell is placed after a measurement chip. Then, it is possible to obtain the image of a cell from the difference in a refractive index. Furthermore, if an antibody, an enzyme, etc. which react to the matter in connection with the metabolic turnover of a cell specifically are fixed, the situation of the metabolic turnover of a cell is also observable.

[0043]

[Effect of the Invention] Since it enabled it to amend distortion of the aspect ratio of the reflected light at the time of SPR measurement to fitness according to the two-dimensional imaging surface plasmon resonance measuring device and measuring method concerning this invention as explained above, the measurement data corresponding to the actual aspect ratio of the quality of a device under test can be obtained correctly.

[0044] Moreover, when a delimiter presents two-dimensional SPR measurement with a metal thin film appropriately, situations, such as an antigen-antibody reaction, a gene and a living thing, and metabolism of a cell, can be measured by many channels.

[Translation done.]

* NOTICES *

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2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

TECHNICAL FIELD

[Field of the Invention] This invention relates the special material in a measured solution to a quantum or the surface plasmon resonance measuring device measured qualitatively, and a measuring method using optical system. Refractive-index change of quality of a device under test, such as a liquid and gas, is detected in detail using the surface plasmon resonance phenomenon in the metal thin film which touched the quality of a device under test, quality and quantum measurement are performed, and it is related with the measuring device which enables two-dimensional measurement of a metal thin film side, and its measuring method.

[Translation done.]

PRIOR ART

[Description of the Prior Art] Conventionally, measurement which used color reaction and an immunoreaction by chemical process measurement, environmental measurement, a clinical laboratory test, etc. is performed. However, it is necessary to carry out the sample extract of the quality of a device under test and also, and there are problems, such as needing complicated actuation and a complicated marker, by such measuring method. On the other hand, the sensor which used the optical-pumping surface plasmon resonance phenomenon by high sensitivity as a possible sensor of the quality and quantum measurement of the chemical in the quality of a device under test is proposed and put in practical use, without needing a marker. Hereafter, surface plasmon resonance (Surface Plasmon Resonance) may be abbreviated to SPR, and may be used.
[0003] As a system which constitutes a SPR sensor, as shown, for example in drawing 1 Incidence of the polarizing plate 2 which lets only p-polarized light light pass for the light emitted from the light source 1, and the condenser lens 3 is carried out with the incident angle in the through quantity refractive-index prism 4. The system which irradiates the metal thin film 5 with the sensor film which touches the quality 6 of a device under test, and detects a change of the reflected light from the metal thin film 5 on the strength with the photoelectron detector 7 through prism 4 is common.
[0004] The light emitted from the light source 1 makes an evanescent wave occur in the interface of prism 4 and the metal thin film 5, and the wave number is defined by the degree type.
kev=kp np sintheta -- here -- kp The wave number of incident light, and np The refractive index of prism and theta are incident angles.
[0005] On the other hand, a surface plasmon wave arises and the wave

number is defined by the front face of the metal thin film 5 by the degree type.

ksp=(c/omega) -root (epsilonn2/(epsilon+n 2))

Here, for c, the velocity of light and omega are [the dielectric constant of a metal thin film and n of angular frequency and epsilon] the refractive indexes of the quality of a device under test.

[0006]

EFFECT OF THE INVENTION

[Effect of the Invention] Since it enabled it to amend distortion of the aspect ratio of the reflected light at the time of SPR measurement to fitness according to the two-dimensional imaging surface plasmon resonance measuring device and measuring method concerning this invention as explained above, the measurement data corresponding to the actual aspect ratio of the quality of a device under test can be obtained correctly.
[0044] Moreover, when a delimiter presents two-dimensional SPR measurement with a metal thin film appropriately, situations, such as an antigen-antibody reaction, a gene and a living thing, and metabolism of a cell, can be measured by many channels.

[Translation done.]

* NOTICES *

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] This invention is made in view of such the present condition, the technical problem is characterized by having the function which amends an aspect ratio, and it is in offering the two-dimensional imaging surface plasmon resonance measuring device with which the measurement data corresponding to the actual aspect ratio of the quality of a device under test is obtained. Furthermore, another technical problem of this invention is to offer the approach of measuring situations, such as multi-channel measurement of biological substances, such as an antibody antigen reaction which used the equipment by this invention, or a gene, a living thing, and a metabolic turnover of the matter of a cell.

[Translation done.]

MEANS

[Means for Solving the Problem] In order to solve the above-mentioned technical problem, this invention adds an aspect ratio amendment function so that the image obtained in a two-dimensional imaging surface plasmon resonance measuring device may be obtained similarly to the aspect ratio of the quality of a device under test. Namely, the two-dimensional imaging surface plasmon resonance measuring device concerning this invention The prism equipped with the metal thin film which touches the quality of a device under test on the front face, and the optical means to which incidence of the light from the light source is carried out from a prism side as the parallel flux of light of p-polarized light light, It has a light-receiving means to measure the two-dimensional light-receiving quantity of light of the reflected light including absorption by the surface plasmon resonance phenomenon from a metal thin film. It is characterized by having a means to amend the distortion to the aspect ratio in the contact surface to the metal thin film of the quality of a device under test of the aspect ratio of the reflected light by carrying out incidence of the flux of light aslant to the field of said metal thin film with said ON light means.
[0013] In aspect ratio amendment, the operation approach which amends the data detected with the photodetector of a light-receiving means using arithmetic units, such as a computer, the thing which amends with an optical means before the reflected light goes into a photodetector, and the mechanical approach made to drive so that the location of the photodetector itself can always be amended can be considered.
[0014]

EXAMPLE

[Example] Hereafter, although an example explains this invention still more concretely, this invention is not limited to these examples.
It measured by producing equipment with structure as shown in example 1 drawing 5 . The light source was set to LED and formed the metal thin film in the glass plate of BK7 of the same refractive index as prism by the spatter. When the break of a metal thin film applied a light-sensitive nature macromolecule to homogeneity on a metal thin film and irradiated ultraviolet rays through a mask, the required part was stiffened, and a part for a non-hard spot was dropped on the organic solvent, and was produced. The pattern is a pattern of the shape of a grid as shown in drawing 12 . The glass plate which carried out the spatter of the metal thin film was stuck on prism in matching oil, and it measured by using quality of a device under test as water. As shown in drawing 18 , although the raw image of aspect ratio unconverted CCD had turned into an image compressed into the longitudinal direction, the image processing was performed by computer and the image with the same aspect ratio as the pattern of the original metal thin film as shown in drawing 19 was obtained. Here, since it is fixing to an incident angle from which surface plasmon resonance takes place with water, the part (part to which water is in contact with the metal thin film directly) without the pattern of a macromolecule has been reflected darkly.
[0033] It measured by producing equipment with structure as shown in example 2 drawing 6 . The light source carried out incidence of the light of LED to the multimode optical fiber, and used the light by which outgoing radiation was transmitted and carried out in the inside of a fiber.

* NOTICES *

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the outline block diagram of the conventional SPR sensor.
[Drawing 2] It is the property Fig. showing an example of the measurement result obtained by the conventional SPR sensor.
[Drawing 3] It is the outline block diagram of the conventional two-dimensional SPR sensor.
[Drawing 4] (a) is a metal thin film pattern used for a two-dimensional SPR sensor, and (b) is the schematic diagram of the measurement result at the time of measuring the above-mentioned pattern by the conventional two-dimensional SPR sensor.
[Drawing 5] It is the outline block diagram of the system which amends the aspect ratio concerning this invention by computer.
[Drawing 6] It is the outline block diagram of a system in case an optical means amends an aspect ratio.
[Drawing 7] It is the outline block diagram of the system in the case of amending an aspect ratio by the mechanical means.
[Drawing 8] It is an outline block diagram at the time of changing the include angle of the system of drawing 7 .
[Drawing 9] It is the outline block diagram showing another gestalt concerning this invention.
[Drawing 10] It is the outline block diagram showing another gestalt in the pan concerning this invention.
[Drawing 11] It is the outline block diagram showing another gestalt in the pan concerning this invention.
[Drawing 12] It is the schematic diagram of the metal thin film pattern used for a two-dimensional SPR sensor.
[Drawing 13] It is the outline sectional view showing the application as a

multi-channel sensor which fixed a different antibody, the enzyme, etc.
[Drawing 14] It is the schematic diagram showing an example of the measurement result of a multi-channel sensor.
[Drawing 15] It is the outline sectional view showing the application as a multi-channel sensor which measures a different sample.
[Drawing 16] It is the schematic diagram showing an applied example as image sensors.
[Drawing 17] It is the schematic diagram of the measurement result when applying as image sensors of drawing 16 .
[Drawing 18] It is drawing which photoed the measurement result in the case of having no aspect ratio translator in an example 1.
[Drawing 19] It is drawing which photoed the measurement result when the computer in an example 1 performs aspect ratio conversion.
[Drawing 20] It is drawing which photoed the measurement result at the time of performing aspect ratio conversion in an example 2.
[Description of Notations]
1 Light Source
2 Polarizing Plate and Polarizer
3 Lens
4 Prism
5 Metal Thin Film
5a The metal thin film which forms a measurement side and which was divided into plurality
5b The data acquisition side observed
6 Sample (Quality of Device under Test)
7 Photoelectron Detector
8 Cel
9 CCD Camera
10 Computer
11 Aspect Ratio Amendment Device
12 Actual Measurement Side
13 Bent Image Observed
14 Amended Image
15 Incident Light Side Arm
16 Outgoing Radiation Light Side Arm
17 Arm
18 Joint Section
19 Arm
20 Transparent Body
21 Divided Metal Thin Film

22 Macromolecule
23 Antibody, Enzyme, Etc.
24 Quality of Device under Test
25 Part Which Fixes Neither Antibody nor Enzyme
26 Part Which Fixed Antibody, Enzyme, Etc.
27 Living Thing and Cell
28 Part Which Living Thing, Cell, Etc. are Metabolizing
29 The Remains of Metabolic Turnover
31 41 Thermal light source
32 35 Polarizer
33, 42, 53 Prism
34 43 Metal thin film
36 55 CCD camera
44 Two-dimensional Image -- Spectrum -- System
51 Semiconductor Laser Light Source
52 Grid-like Laser Beam

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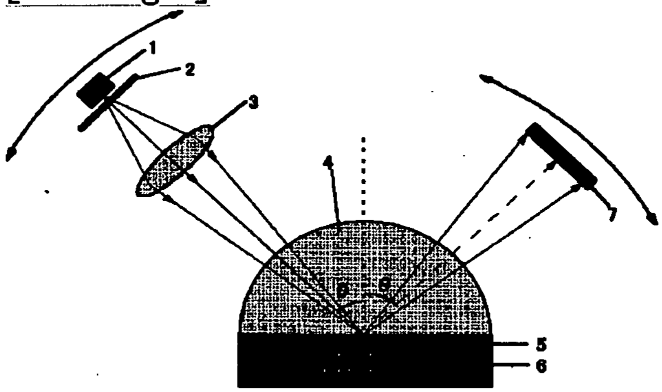
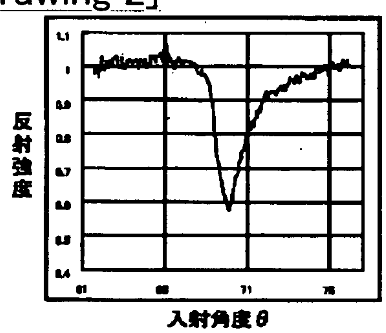
DRAWINGS

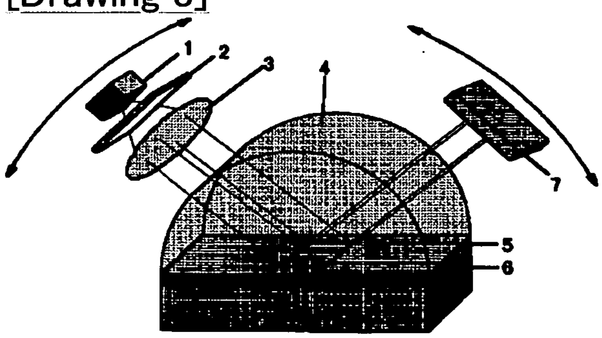
[Drawing 1]



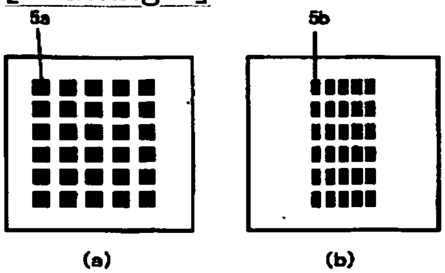
[Drawing 2]



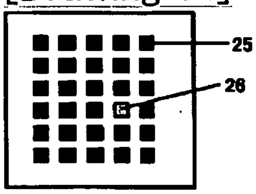
[Drawing 3]



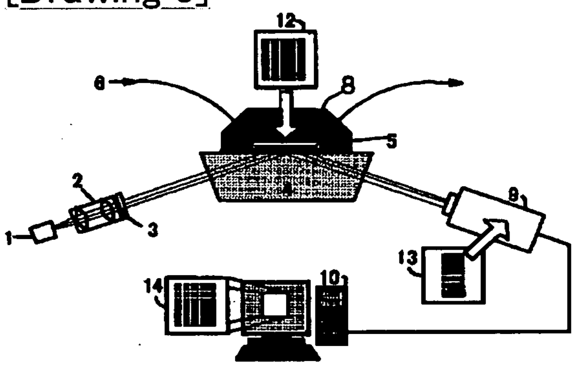
[Drawing 4]



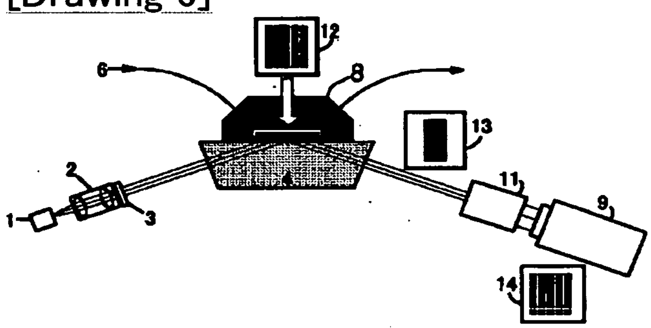
[Drawing 14]



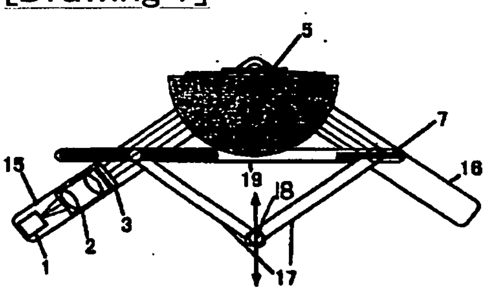
[Drawing 5]



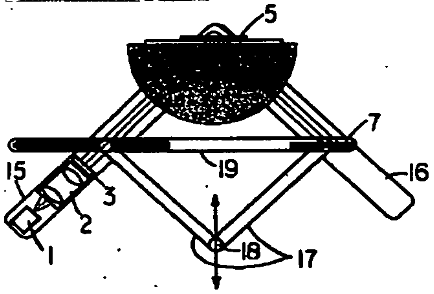
[Drawing 6]



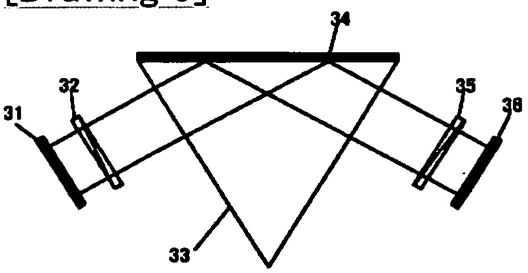
[Drawing 7]



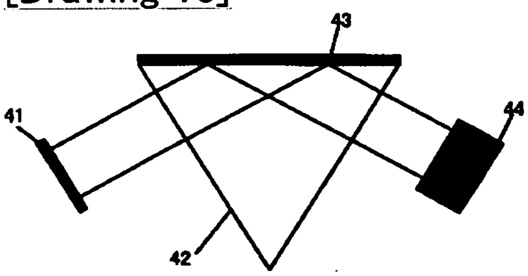
[Drawing 8]



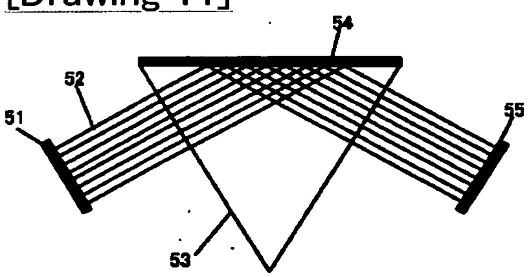
[Drawing 9]



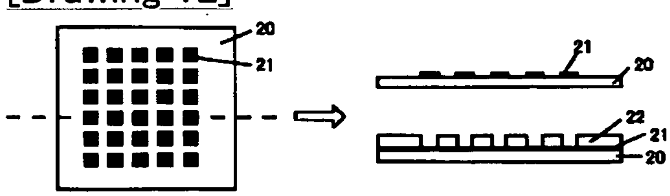
[Drawing 10]



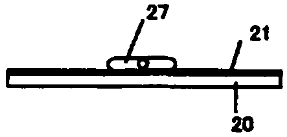
[Drawing 11]



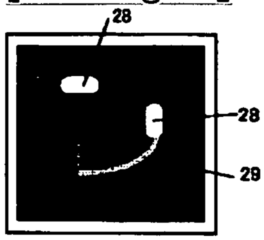
[Drawing 12]



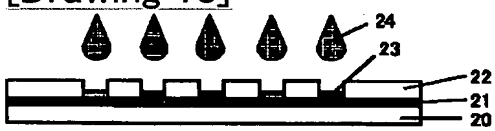
[Drawing 16]



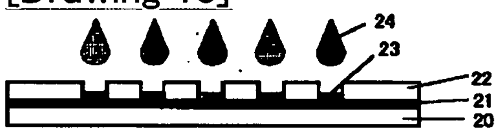
[Drawing 17]



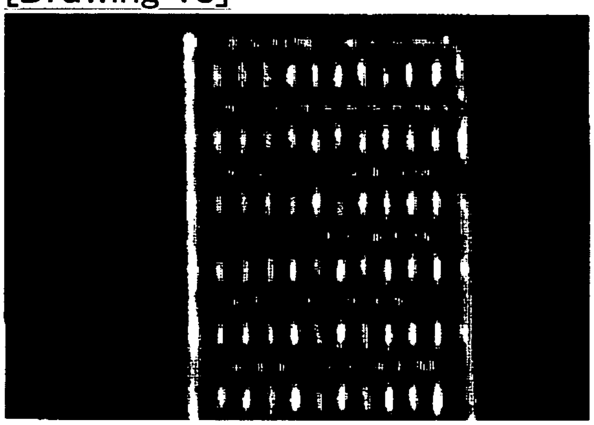
[Drawing 13]



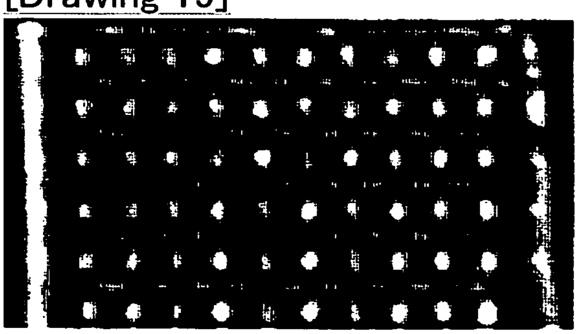
[Drawing 15]



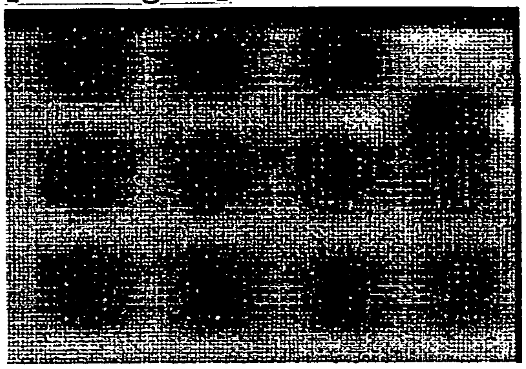
[Drawing 18]



[Drawing 19]



[Drawing 20]



[Translation done.]